Chronic and Recurrent Diarrhea in American Servicemen in Vietnam

An Evaluation of Etiology and Small Bowel Structure and Function

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Seventy American servicemen in Vietnam had diarrhea of at least two weeks’ duration with no evidence of infection with Entamoeba histolytica or Shigella species. The majority had malabsorption as shown by D-xylose absorption, fecal fat excretion, and results of Schilling tests. Jejunal biopsy revealed jejunitis in ten patients; their D-xylose absorption and vitamin B12 excretion levels were significantly lower than those of patients without jejunitis. Fourteen patients had bacterial contamination of the jejenum, and 11 patients harbored the intestinal helminths hookworm or Strongyloides stercoralis; Giardia lamblia was present in 25 patients who had more severe jejunitis and malabsorption than patients without G. lamblia. The frequent occurrence of intestinal parasites and the favorable responses in 91% of patients to regimens that included appropriate antiparasitic drugs suggests that intestinal parasitosis, especially giardiasis, was a common cause of diarrhea in these patients.

The threat of diarrhea to a visitor in tropical countries from temperate zone countries is a well-known problem. The American serviceman with field duty in Vietnam is an extreme example of a visitor in the tropics who often must ingest unprocessed water and uncooked foods. Because chronic and recurrent diarrhea with weight loss was a disabling disease frequently seen at the US Naval Support Activity Station Hospital, Danang, Republic of South Vietnam, we sought to clarify its causes and characterize its effect on the structure and function of the small intestine.

Patients and Methods

Seventy previously healthy American soldiers in Vietnam ranging in age from 18 to 28 years were studied. They had been serving in the field from 2 to 18 months, where they frequently drank unprocessed water from wells and streams and occasionally ate Vietnamese food in villages. All had experienced nonbloody, watery, or semisolid diarrhea for periods of two weeks to ten months before admission. All had diarrhea at the time of admission. Most had experienced recurrent episodes. Weight loss ranging from 2.3 to 19.8 kg accompanied the diarrhea. In all instances, there had been attempts at therapy in the field with constipating agents, antibiotics, which were commonly tetracycline or a mixture of dihydrostreptomycin sulfate and polymyxin sulfate (Polymagma), or various antiparasitic drugs. To exclude acute diarrheal diseases, all candidates for the study underwent sigmoidoscopy and those showing colitis, mucosal ulcers, or trophozoites of Entamoeba histolytica were excluded. Also excluded were patients showing E histolytica cysts in subsequent stool specimens or having positive cultures for Shigella or Salmonella species. Except for hyperactive bowel sounds, findings of physical examinations were normal. No one had fever. A control group of 17 men of similar age and field duty who did not have diarrhea were also studied.

In the fasting state, an alcohol-sterilized polyethylene tube with mercury bag was passed through the nose. At a position just beyond Treitz' ligament an aspirate of jejunal contents was taken. After flushing the tube with sterile water it was allowed to pass 220 cm from the nose where another aspirate, considered to be ileal contents, was taken. The aspirates were serially diluted in saline and inoculated on blood agar (Salmonella-Shigella agar), eosin methylene blue agar, and chocolate agar. The chocolate agar plates were incubated in an anaerobic jar.

Fresh saline preparations for parasites were made at the time of both sigmoidoscopy and small bowel aspiration. Three separate stool specimens from each patient were concentrated by the method of zinc sulfate centrifugal flotation and examined for ova in iodine solution. Touch preparations of mucus from jejunal biopsy specimens were stained with Giemsa stain for Giardia lamblia. Rectal swabs and small bowel aspirates were cultured on HEP-2 and monkey kidney cell lines for enteroviruses.

Absorption of a 25-gm dose of D-xylose was determined by measurement of the two-hour blood level and five-hour urinary excretion. Mean values of the control subjects were 34.7 mg/100 ml and 7.2 gm/100 ml, respectively. Initially the D-xylose absorption was the only absorption test available to us. Later in the course of study, fecal fat and Schilling tests became available and were performed on smaller numbers of patients. The fecal fat excretion was measured on a pooled three-day collection. A normal value was considered to be less than 6 gm/day. Vitamin B12 absorption was measured by the Schilling test with intrinsic factor. Mean value for the controls was 9.2% excretion. Serum immunoglobulin IgA, IgG, and IgM levels were estimated by radial immunodiffusion.
Jejunal biopsies were obtained with a Crosby-Kugler capsule. Sections were stained with hematoxylin and eosin. Slides were read separately by the authors and independently by the staff pathologist who had no knowledge of the other data. Jejunal inflammation was assessed on a 0 to 4 scale based on villous architecture, columnar cell height, and lamina propria infiltrate. The five reader’s grades were averaged for each patient.

Therapy was not controlled as a part of this study. By preference of the hospital staff, all patients received a mixture of diphenylxylate hydrochloride and atropine sulfate (Lomotil) or kaolin mixture with pectin (Kaopectate). When parasites were identified, the drugs tetrachloroethylene, thiabendazole, quinacrine hydrochloride, or metronidazole were used appropriately. In most patients with jejunitis or severe malabsorption, tetracycline and folic acid were used. In no case was tetracycline given before intestinal aspirates for culture were obtained.

Results

Small Bowel Cultures.—Adequate material for culture was obtained from 52 patients and eight controls. Considering a concentration of 10⁴ colonies per milliliter as suggesting significant growth or contamination, we found that 14 patients (27%) and two controls had bacterial contamination in the jejunum. In contrast, 31 patients (60%) had 10⁴ organisms or more in the ileum, as did six controls. The bacteria isolated and the frequency of isolation are shown in Table 1.

Virus Cultures.—All attempts at enterovirus culture from the 70 patients gave negative results.

Parasite Studies.—The following parasites were found in the patients: *G. lamblia* in 25, hookworm in ten, *Strongyloides stercoralis* in three, *Entamoeba coli* in three, *Endolimax nana* in two, and *Isospora belli* in one. Eight patients had two or more parasites. Although *G. lamblia* cysts were seen in stools of 25 patients, only three of these had motile trophozoites in their stools. Trophozoites were found in the biopsy touch preparations of five patients, and in three of these the diagnosis of giardiasis was made prior to finding cysts in the stool. Adult hookworms were recovered from the small bowel of only one of ten patients with stool ova. The three patients with *S. stercoralis* had larvae in the small bowel but none had ova in the stool.

Absorption Tests.—D-xylose excretion was less than 5.0 gm in 40 of 64 patients tested (63%). Twenty-four of these had two-hour blood values less than 30 mg/100 ml. The mean D-xylose excretion for all patients was 4.98 gm and the mean blood D-xylose level was 25.6 gm/100 ml. Fecal fat excretion was measured during a period of regular diet and was elevated in 21 of 26 patients tested, with a mean of 12.7 gm/day. The Schilling test result was abnormal in 14 of 29 patients tested (48%), and the mean excretion was 9.1%.

Immunoglobulins.—The serum immunoglobulin values were normal in 29 of 30 patients tested. One patient with giardiasis had a low IgA level of 30 gm/100 ml.

Biopsies.—Adequate biopsy material for grading was obtained from 59 patients and 14 controls. The results of most biopsies were graded as normal or minimally changed. However, biopsies from patients with diarrhea when compared to those of the controls more frequently showed nonspecific inflammatory changes of villus shortening and coalescence, crypt cell hyperplasia, and round cell infiltrate of the lamina propria. Table 2 shows the distribution of severity of these changes seen in the biopsies. None was graded as severe or "sprue-like." Examples of biopsy changes and grading are shown in the Figure.

The ten patients with biopsy grades 2 and 3 were considered to have jejunitis, while those with biopsy grades 0 and 1 were considered not to have jejunitis. Table 3 shows the mean values of clinical and laboratory data in each biopsy category. Both groups had essentially the same duration of diarrhea, time in Vietnam, weight loss, and fecal fat excretion. The mean D-xylose values and vitamin B₁₂ excretion were considerably lower in the patients with jejunitis. Student’s *t* test revealed that differences in both the blood D-xylose values and vitamin B₁₂ excretion were statistically significant (*P*<.05).

Comparison of Intestinal Bacterial Counts and Parasitology With Other Data.—When the 14 patients with jejunal bacterial contamination were compared to patients with low bacterial counts in respect to absorption tests and biopsy grades, no differences were found. Similarly, the 11 patients with helminths were compared to patients without helminths, and no differences were present. Only one patient with jejunitis had hookworm, and one had *S. stercoralis*. In contrast, infection with *G. lamblia* was associated with jejunitis and malabsorption. Seventy percent of the patients with jejunitis harbored *G. lamblia* while only 29% of those without jejunitis had this parasite (*P*<.05). D-xylose excretion in patients with *G. lamblia* was less than that in patients without it, 4.0 gm compared with 5.4 gm, but this difference was not statistically significant (*P*>.05).

Course and Treatment.—Ninety-one percent (64 of 70) of all patients became asymptomatic during the first
Jejunal biopsy Grades (hematoxylin and eosin, original magnification ×90). Top left, Grade 0 or normal; tall, slender villi. Bottom left, Grade 1 or minimal; shortened villi and crypt enlargement. Top right, Grade 2 or mild; thickened villi and cellular infiltration of lamina propria. Bottom right, Grade 3 or moderate; flattened villi, elongated crypts, and heavy cellular infiltrate.

week of hospitalization regardless of therapy employed. Only six patients (9%) were considered to have treatment failures. Eleven patients harboring helminths received either tetrachloroethylene or thiabendazole, and all showed improvement. Of ten patients with giardiasis who were treated with quinacrine hydrochloride, 100 mg three times a day, eight showed rapid improvement and two had no response during two weeks of therapy. Three patients with jejunitis who were treated with tetracycline failed to show improvement over a period of three weeks and were evacuated from Vietnam. The remaining seven patients with jejunitis who were treated with either tetracycline or antiparasitic drugs showed improvement and were returned to field duty. One of these had a relapse a month later, failed to respond to tetracycline and folic acid therapy, and was evacuated.

**Comment**

The patients in this study were highly selected. To be evacuated from field duty they had to show symptomatic treatment failure of at least two weeks' duration, often with histories of recurrent diarrhea, and in many instances they had been blindly treated with antibiotics and antiparasitic drugs. In patients admitted to this study, two common causes of acute diarrhea in this population, shigellosis and amebiasis, were excluded. These patients, therefore, represent one end of the spectrum of diarrheal illness in Americans serving in Vietnam and must be clearly separated from the acute syndromes of travelers to tropical countries.

A striking feature of these patients was the high incidence of malabsorption. Less commonly seen was jejunitis—14% of patients. However, these patients with jejunitis had significantly more severe abnormalities of absorption studies than did patients without jejunitis. This correlation of structure and function of the small bowel conforms with findings in other studies of tropical diarrhea and agrees closely with results of another study of jejunal morphology in Americans in Vietnam in which jejunitis was reported in 22 of 75 recent
E. coli isolated from Americans in the United States was seen in anemia, deficiencies leading to malnutrition and dietary restrictions themselves producing symptoms. The definition of tropical sprue is "telescoping" of the bowel. Most of our patients with diarrhea in Vietnam were able to be considered to have "acute tropical sprue" by current definitions. However, the usual features of weight loss, lassitude, and malabsorption were complicated by the fact that strenuous field duty and dietary restrictions themselves produced similar symptoms. Vitamin deficiencies leading to megaloblastic anemia, seen in classical tropical sprue, did not develop in the relatively short duration of diarrhea in our subjects.

Attempts to define specific causes of the diarrhea were fruitful in many patients. About one half of the patients harbored parasites, in particular the helminths and G. lamblia, which may be diarrheal pathogens. In the remainder no causes could be ascertained. However, certain helpful conclusions can be drawn from the results of these studies. The negative virus cultures rule out enteroviruses as a cause of the diarrhea. This finding is in accord with other negative results in attempts at virus isolation in tropical sprue.

Results of our bacterial cultures suggest that bacterial contamination of the upper jejunum was not frequently associated with diarrhea. However, our aspirates were taken high in the jejunum near the Treitz' ligament, and we may have missed contamination in the midjejunum. Ileal samples showed heavy growth in most cases, but were taken at 220 cm from the nose at a position that was not roentgenographically verified and may have been at the caecum because of "telescoping" of the bowel on the polyethylene tube. Most of our ileal specimens from controls also showed contamination. The role of small-bowel bacteria in producing tropical diarrhea remains uncertain. Studies of tropical sprue in India have shown heavy contamination in the upper part of the small bowel while other workers have reported lack of contamination in patients with sprue.

Limitations of our field laboratory restrict interpretation of our results. Escherichia coli isolated from our patients were not serotyped or evaluated for pathogenicity. Others have shown that E. coli isolated from patients with diarrhea in Vietnam were capable of producing either a Shigella-like colitis or a cholera-like diarrhea in human volunteers. However, in our patients the normal findings of sigmoidoscopy rule out colitis, and the presence of jejunitis in some patients rules out a purely cholera-like mechanism because the latter should not produce inflammatory changes in the mucosa.

Strict anaerobic handling of specimens could not be achieved, and our isolates were largely aerobic organisms. Improved anaerobic culture techniques have revealed that obligate anaerobes comprise most of the human intestinal flora. A role of anaerobic bacterial overgrowth in producing diarrhea in our patients cannot be excluded.

Intestinal parasites were believed to be a major cause of diarrhea in our patients. A previous study of diarrhea in American soldiers in Vietnam showed that 11.8% of patients had intestinal parasites by drinking unprocessed water from streams, wells, and even rice paddies that are contaminated with both human and animal excreta. It was a distinct impression of our hospital staff that soldiers with field duty were highly susceptible to diarrhea, while personnel in base camps were relatively safe. This experience is analogous to studies of Peace Corps volunteers in Pakistan who more commonly had diarrhea, malabsorption, and intestinal parasites than did Americans not exposed to local food and water.

The helminths, hookworm, and S. stercoralis were found in 11 patients and may have been responsible for their diarrhea. Our patients with helminths, however, did not have more malabsorption or more severe jejunitis than other patients. Some investigators have shown these helminths to be associated with malabsorption in tropical countries, but other workers disfavor the hypothesis of hookworm as a factor in producing malabsorption.

Patients with G. lamblia were more frequently had jejunitis and malabsorption than patients without this parasite. It was considered a probable cause of diarrhea in most of the 25 patients harboring it. Although G. lamblia may be carried as an intestinal commensal in asymptomatic people, it has been firmly established as a diarrheal pathogen capable of producing malabsorption. It can cause diarrhea in previously healthy persons and has commonly been associated with underlying illnesses, particularly dysgammaglobulinemia. In our patients, the association between giardiasis and jejunitis contrasts with the findings of others that healthy Americans in the United States infected with G. lamblia have normal results in jejunal morphologic studies. Patients with immunoglobulin deficiencies, on the other hand, will show jejunitis during G. lamblia infection. None of our patients with giardiasis had evidence of underlying immunologic disease, although one man did have low serum IgA level. Because tropical sprue is endemic in Vietnam it must be considered that the jejunitis in our patients with giardiasis represented early changes of tropical sprue. It is conceivable that G. lamblia trophozoites act synergistically with the etiologic mechanisms of tropical sprue.

| Table 3.—Mean Values of Clinical and Laboratory Data in Diarrhea Patients With and Without Jejunitis |
|--------------------------------------------------|---------------------|---------------------|
| Measurement                                      | Jejunitis           | No Jejunitis        |
| Duration of diarrhea, mo                        | 1.9                 | 1.6                 |
| Time in Vietnam, mo                            | 6.8                 | 6.5                 |
| Weight loss, kg                                 | 8.6                 | 8.1                 |
| D-xylene excretion, gm                          | 3.0                 | 5.2                 |
| D-xylene two-hour blood level, mg/100 ml        | 13*                 | 28                  |
| Fecal fat excretion, gm/day                     | 10                  | 14                  |
| Vitamin B<sub>12</sub> excretion, % of dose     | 3.0*                | 10.5                |

* Differences statistically significant by t test, P < .05.
which are poorly understood, to produce mucosal injury in these patients.

Giemsas staining of the biopsy mucos or of small bowel aspirates was an effective approach to the diagnosis of giardiasis in this study and may yield the diagnosis in the presence of negative results in stool examinations. In contrast, the hematoxylin and eosin stains of the biopsies provided a poor yield because this stain is not optimal for G lamblia. Mucosal invasion by G lamblia was not observed in these patients as described by others, but it cannot be ruled out because Giemsas stain was not applied to our biopsy sections.

Prompt resolution of diarrhea in 91% of these patients after a week in the hospital regardless of diagnosis or therapy was a prominent feature among our patients. This may suggest that mere removal from field duty and return to more comfortable surroundings and a hospital diet were responsible for their improvement.

Symptomatic therapy with antisapmodics and constipating agents played an unknown role. On the other hand, the success of the various treatments used may be partially the result of antiparasitic drugs directed specifically against the parasites that were found. All patients with helminths showed improvement as did most of the patients with giardiasis treated with quinacrine hydrochloride. Most of the patients who had treatment failures had significant jejunitis.

Some of the diagnostic measures used in this study may be useful to others evaluating similar symptoms in patients for therapy. Sigmodioscopy, multiple stool examinations, and jejunal intubation uncovered parasitic infections in about half of our patients that were considered therapeutically important. Results of the absorption studies were frequently abnormal and were not useful for selecting treatment. However, jejunal biopsies might be recommended in patients who do not promptly show improvement with symptomatic or antiparasitic therapy for two reasons: (1) jejunitis may define a population of patients with diarrhea more likely to have prolonged courses or require evacuation from the tropical country, and (2) jejunitis associated with weight loss and malabsorption may be considered tropical sprue, for which treatment regimens including folico acid, vitamin B12, or tetracycline have been shown to be effective."

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John Aussem, MD, reviewed the slides. Statistical analysis was performed by C. C. Lin and Richard See. John H. Yardley, MD, photographed the biopsy specimens.

Nonproprietary Name and Trademark of Drug

Metronidazole—Flagyl

References